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Antimutagenesis in Microbial Systems

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INTRODUCTION

Our reasons for writing this review are threefold. First, we are currently working in this field and believe that this area of study has been comparatively neglected, yet is intrinsically as interesting and important as a study of mutagenesis. We hope this review will stimulate others to solve some of the many remaining problems in this area. Second, we know of only two reviews dealing with this topic. One of these dates from 1956 (119); the other, although more recent, covers only antimutagenic effects against spontaneously arising antibiotic-resistant mutants in bacteria (35). Third, one cannot understand the whole process of mutagenesis, including the regulation of mutation frequencies and mutagen specificities, without considering antimutagenic effects. Of particular interest are the interrelationships that are emerging between antimutagenic action, repair systems, and other processes such as genetic recombination. Furthermore, environmental antimutagenesis may turn out to be a powerful tool to control environmental mutagenesis.

We shall begin by defining some terms. A pre-mutation is a lesion in the deoxyribonucleic acid (DNA) which is potentially able to give rise to a mutation. Whether this potential is realized will depend on various factors such as the activity of repair systems and successful passage through the intricacies of the other aspects of the *mutational pathway* (see below). We define a mutation as a stable, heritable change in the DNA. This will result from an altered configuration of one or more base pairs. A mutation may exist in a cell that does not yet exhibit mutant phenotype. A mutant is an individual organism that exhibits a mutant phenotype (i.e., in which mutational expression has been achieved). These distinctions are well illustrated in the case of ultraviolet (UV)-induced mutations to streptomycin resistance. Here pyrimidine dimers are premutational lesions; mutations in the DNA are subsequently formed by alterations in base pair sequences, but phenotypic expression delay, involving cell divisions and synthesis of altered ribosomal proteins, precedes final production of mutants which are phenotypically streptomycin resistant.

It is essential that we define clearly what we mean by antimutagenesis. True antimutagenesis will involve any agent or effect that specifically or preferentially reduces the yield of mutants. Any effects observed should not be merely direct consequences of altered survival (e.g., cell death) or dose reduction (i.e., move-

ment back along a normal dose-response curve for mutation induction). These latter effects we would call apparent antimutagenesis. For example, decreases in absolute numbers of mutants resulting from cell death are not true antimutagenesis, and more importantly, nor are reductions in the yield of mutants brought about by, for example, photoreactivation, if this is a predictable consequence of the observed rise in survival levels. Naturally, in the case of UV mutagenesis occurring at 100% survival, one could argue that photoreactivation-induced reduction in mutational yield was truly antimutagenic since no increase in survival is possible. Thus, a distinction between true and apparent mutagenesis may sometimes be difficult in practice. This illustrates the vital importance of determining complete dose-response curves before concluding that any decreases in mutational yield which are observed are the result of true antimutagenesis (40).

Mutations are generated, processed, and expressed via a complex mutational pathway (5, 22, 26). Thus, mutations may arise, or premutational lesions be generated, at least in theory, at the levels of: (i) reaction between a mutagen and DNA; (ii) the utilization of mutagen-altered precursors or base-analogues in DNA replication or repair; (iii) through errors in DNA replication; (iv) through errors in DNA recombination; (v) through errors in DNA repair; (vi) indirectly via errors in transcription; (vii) indirectly via errors in translation. Mutations arising by mechanisms vi and vii do so indirectly via mechanisms iii, iv, or v, that is to say, the activity of error-prone enzyme molecules (98, 107, 150). Just as there is more than one origin for mutations, so too one should expect antimutagens to have many possible modes of action at various stages of the mutational pathway. For example, antimutagenic effects could arise through interference with the reaction of a mutagen with DNA at the levels of repair, replication, recombination, transcription, translation, phenotypic expression including segregational delay, or, the final step in the mutational pathway, the growth of a single mutant individual into a visible, scoreable, mutant clone. We shall not, therefore, confine our discussion of antimutagenesis to agents that are known or believed (often on the basis of little experimental evidence) to act at any particular step in the mutational pathway.

In this review we shall deal with antimutagenesis from two main standpoints: first, antimutagenesis at what we shall call the physiological level, by which we mean the reduction of mutational yields brought about by addition of chemicals or alteration of cellular conditions; second, antimutagenesis at what we shall call the genetic level, by which we mean antimutagenic effects of alleles in replication genes, repair genes, or the general genetic background. We are well aware that this is essentially a description of antimutagenesis at an observational level and that well-proven biochemical explanations for either genetic or physiological antimutagenesis are presently exceptionally rare. If our review serves to stimulate experimentation into the biochemical bases of antimutagenesis, it will, we believe, have justified its existence.

One of the main points we shall try to bring out throughout this review is the occurrence of antimutagenic specificity. Just as mutagens may exhibit specificities with regard to the types of lesions they induce (transitions, transversions, frameshifts, deletions, hot-spot patterns within single genes, or differential mutability of various genes), so too are antimutagens often observed to affect specific classes of mutations or to antagonize certain mutagens. The analysis of the modes of antimutagenic action and specificity bids fair to become one of the more fascinating aspects of mutation research in the next decade.

PHYSIOLOGICAL ANTIMUTAGENESIS

Purine Nucleosides (Adenosine, Guanosine, and Inosine)

The purine nucleosides adenosine, guanosine, and inosine were the first microbial antimutagens to be discovered (121). In these and subsequent studies with tryptophan-limited *Escherichia coli* B and B/r cells growing at relatively slow rates in chemostats (119), forward mutations to phage T5 or T6 resistance were scored. Under these (but not other) conditions (88), mutation rates are proportional to time but not to the replication rate. Against the purine-induced, replication-independent mutations studied in these experiments, purine nucleosides are particularly effective antimutagens. Adenosine (0.4 μg/ml) or guanosine or inosine (2 μg/ml) gave a 50% decrease in the mutation rate induced by theophylline (150 μg/ml). Adenosine at 50 μg/ml completely abolished the mutagenicities of the purine mutagens caffeine, theophylline, paraxanthine, theobromine, azaguanine, 8-methoxycaffeine, and adenine (at 150 μg/ml). In contrast, even at the higher concentration of 500 μg/ml, adenosine caused only a partial abolition of spontaneous mutations and of the mutagenicities of tetramethyluric acid and benzimidazole. Adenosine had no an-

timutagenic effect on UV- or gamma-induced mutations in tryptophan-limited cells.

In contrast to the above results, tryptophan-limited cells in a rich medium, at short generation times in chemostats, show both replication-dependent and replication-independent spontaneous mutagenesis (52, 88). Adenosine or growth under anaerobic conditions (119) is reported to have no antimutagenic effect on the replication-dependent spontaneous mutability in such tryptophan-limited cells.

Further confirmation of the antimutagenicity of adenosine was provided by Glass and Novick (57). They showed that adenosine (100 μg/ml) completely abolished the mutability by caffeine at 200 μg/ml in both growing and chloramphenicol-inhibited *E. coli* B/r.

The results of Kubitschek and Bendigkeit (89) and Kubitschek (87) with glucose- or tryptophan-limited cells of *E. coli* in chemostats show that purine ribonucleosides are antimutagenic against spontaneous and 2-aminopurine-induced mutagenesis (scoring T5-resistant mutants). There was, however, no reduction in UV-induced mutagenesis, suggesting that purine ribonucleosides act only against mutations arising during DNA replication. However, in the absence of additional information regarding the molecular nature of T5-resistant mutations arising spontaneously or induced by 2-aminopurine or UV light, such a conclusion is necessarily tentative. Moreover, Nestmann (117), using glucose-limited *E. coli* B/r cells in chemostats, where mutation rates are solely replication dependent (88), showed that guanosine (50 μg/ml) caused a 36% reduction in spontaneous and mutator H1-induced T5-resistant mutation frequencies.

In the absence of knowledge about the molecular nature of the $T5^R$ and $T6^R$ mutants studied and of the comparative mechanisms of replication-dependent and -independent mutagenesis, it is very difficult to speculate meaningfully about purine nucleoside antimutagenesis. It may be significant, however, that adenosine is a more effective antimutagen than guanosine or inosine at the same molar concentration (119). One is tempted to believe that replication-dependent mutagenesis probably solely reflects errors in DNA replication and that replication-independent mutagenesis may reflect the activity of error-prone repair systems, perhaps acting upon spontaneously arising single-strand nicks. It would be extremely interesting to determine the effects of known repair deficiencies, e.g., Uvr^-, Rec^-, Lex^- (Exr^-), Pol^-, upon replication-dependent mutagenesis, replication-independent spontaneous mutagenesis, purine-induced mutagenesis, and purine nucleoside antimutagenesis in glucose- and tryptophan-limited chemostat populations, respectively.

One very speculative possibility is that purine nucleosides act to prevent the induction of an error-prone, and hence mutagenic, repair system which is induced in response to lesions in the DNA. Thus, Witkin (165) has recently reported a special case of antimutagenesis by cytidine plus guanosine (100 μg/ml each) or by pantoyl lactone. Cytidine plus guanosine (or pantoyl lactone) prevented the enhanced UV mutability exhibited at low UV dosages in a *tif*$^-$ *E. coli* strain at 42 C. In this strain a set of functions is induced at 42 C (14, 83).

Caffeine

Caffeine is not only a mutagen (57, 120) and repair inhibitor (108, 157) under some conditions in some bacterial systems, but also, under other circumstances, an antimutagen. Grigg and Stuckey (64) studied spontaneously arising $His^- \rightarrow His^+$ revertants in an *E. coli* 15 auxotroph when cells were held in liquid minimal medium over extended time periods. Unfortunately, the molecular nature of this particular His^- mutant and the His^+ revertants is not known, i.e., whether base-pair substitutions or frame-shifts, and their suppressors, were involved. In such nonreplicating stationary-phase cells caffeine, at a concentration of 1,150 μg/ml, caused a greater than 90% reduction in the spontaneous rate of $His^- \rightarrow His^+$ mutation. However, this same concentration of caffeine had no effect on the phenotypically similar, although not necessarily genetically identical, His^+ mutations arising during growth and replication of such His^- cells. These differences in caffeine effect possibly could be due to repression-derepression differences at the *his* or suppressor loci between stationary and growing cells. Alternatively, and more likely, different mechanisms of mutagenesis might operate in stationary and growing cells and, hence, presumably on nonreplicating or replicating DNA. Of particular interest would be a comparison of caffeine antimutagenesis in this stationary-phase system using pairs of otherwise isogenic strains, differing only in single repair functions specified by *exr*, *pol*, *rec*, and *uvr* loci. It may be significant that, whereas caffeine concentrations of 350 to 500 μg/ml cause maximal inhibition of excision repair (139, 143), in their study Grigg and Stuckey (64) observed that caffeine antimutagenesis was totally absent at a concentration of 260 μg/ml. Thus, it may be some type of error-prone repair, other than normal excision repair, that is causing stationary-phase

mutations and is inhibited by higher concentrations of caffeine.

Caffeine antimutagenesis against UV-induced mutations in various *E. coli* strains has been observed in several studies. Horneck-Witt and Kaplan (77) showed that addition of caffeine, at a concentration of 1,000 μg/ml or higher, to the post-irradiation broth incubation medium caused a decrease in the yield of UV-induced mutations conferring low-level (3 μg/ml) resistance to streptomycin. These experiments were performed in an *E. coli* B derivative, B/*phr*$^-$/MC2.

Clarke (21) using UV-irradiated, broth-grown stationary-phase cells of an Hcr$^-$ (*uvrA*) derivative of *E. coli* B/r *trp*$^-$ WWP-2 showed that 500 μg of caffeine per ml added to the amino acid-enriched minimal plating medium caused a small, but real, decrease in the yield of UV-induced Trp$^-$ → Trp$^+$ reversions. Many of these Trp$^+$ revertants result from ochre suppressor mutations. Results similar to those described above but in which there was scoring of UV-induced mutations to high-level (1,000 μg/ml) dihydrostreptomycin resistance (Str^R) were obtained by Sideropoulos and Shankel (144) in *E. coli* B/r WWP-2 Hcr$^-$. Their data revealed a consistent diminution of induced mutational yields in the presence of 500 μg of caffeine per ml.

Witkin and Farquharson (166) made an extensive study of caffeine effects upon UV-induced mutational yield in repair-proficient and -deficient strains of *E. coli*. Mutations studied were those from streptomycin sensitivity to resistance (Str^R) in an excision repair-defective (Hcr$^-$) strain and mutations from auxotrophy to prototrophy in both Hcr$^+$ and Hcr$^-$ strains. Broth-grown late-lag-phase cells were used in these experiments, and caffeine concentrations of 1,000 and 2,000 μg/ml were employed. The high concentration of caffeine consistently depressed the yield of induced mutations of both types in all Hcr$^-$ strains, particularly at low doses of UV. In an Hcr$^+$ strain, caffeine at 2,000 μg/ml caused a smaller enhancement of UV-induced Trp$^-$ → Trp$^+$ reversions than did a 1,000 μg/ml concentration. This result is consistent with the idea that in an Hcr$^+$ strain the enhancing effect of caffeine (resulting from inhibition of excision repair) is to some extent counteracted by an opposing antimutagenic effect of caffeine. Unfortunately the generality of this result cannot be assumed since in their paper Witkin and Farquharson did not report on results with high concentrations of caffeine in an Hcr$^+$ strain, scoring mutations from streptomycin sensitivity to resistance. However, they showed that caffeine can exert its antimutagenic action on the UV-induced yield of prototrophic revertants in an Hcr$^-$ strain up to the time when the first postirradiation replication occurs. Furthermore, in matings between *E. coli* K-12 Hfr Cs101 *met*$^-$ *str*$^+$ strain and three different *E. coli* B Hcr$^-$ Str^R auxotrophic strains, a concentration of 2,000 μg of caffeine per ml in the final plating medium, after 100 min of mating, caused a 50 to 70% diminution in recombinant yield. Although these results may well indicate that high concentrations of caffeine interfere with genetic recombination, one cannot rule out the possibility that the observed reductions in recombinant yields were due to other causes. For example, in the above matings, which involve K-12 × B crosses, restriction phenomena will certainly operate. Thus, these interesting results need to be confirmed in Hfr × F$^-$ crosses involving strains that do not differ in restriction-modification patterns. The fact that in Hcr$^-$Exr$^-$ strains there was no enhancement of UV-induced killing by even high concentrations of caffeine lead Witkin and Farquharson to postulate that the effect of caffeine is to inhibit both the activity and error-proneness (and hence mutagenicity) of the Exr system. They suggested that this effect could be either direct or indirect by an inhibition of nuclease action. They further predicted (results of such experiments have not yet been reported) that high concentrations of caffeine should be antimutagenic in Hcr$^-$ strains against gamma-ray and thymineless mutagenesis, since the mutability of these agents, as with UV, is supposed to depend on errors in recombinational postreplication repair (12). This topic will be dealt with in more detail when the effects of *exr* and *rec* mutations on mutability are discussed below.

With lag- and exponential-phase cells of *Salmonella typhimurium* grown before irradiation in liquid minimal medium plus tryptophan (an essential auxotrophic requirement), Williams and Clarke (155) were able to demonstrate an antimutagenic effect of caffeine (500 μg/ml). Caffeine, when added to a post-irradiation plating medium devoid of an amino acid mixture (acid-hydrolyzed casein), reduced the yield of UV-induced Trp$^-$ → Trp$^+$ reversions. Reconstruction experiments excluded a selective action against growth of established Trp$^+$ cells into scoreable colonies. Caffeine antimutagenesis resulted neither when a plating medium supplemented with casein hydrolysate was used, nor when cells grown before irradiation under other physiological conditions were employed.

There is evidence that caffeine is antimuta-

genic in *E. coli* not only against UV-induced mutations, but also against those induced by nitrous acid. In the excision repair-deficient *uvrA⁻trp⁻ E. coli* B/r strain WWP-2 Hcr⁻, Clarke (25) showed that, regardless of whether comparisons were made at equal survival or equal dose levels, caffeine at 500 μg/ml had an antimutagenic action. This was in contrast to an enhancing effect of caffeine on the Trp⁺ revertant yield in the Hcr⁺ excision-proficient strain. Tentatively this result may indicate an inhibition by caffeine of DNA replication or postreplication error-prone processes that play a positive role in nitrous acid mutagenesis. There is evidence from transformation and transfection experiments with *Bacillus subtilis* (106) and phage KBl-mediated transduction in *S. typhimurium* (Clarke, unpublished data) indicating that caffeine inhibits recombination processes.

Caffeine, at a concentration of 1,000 μg/ml, has been found to be an antimutagen against both spontaneous and 2-aminopurine-induced Trp⁻ → Trp⁺ reversions, resulting from both back-mutations of an ochre codon and ochre suppressors, in *E. coli* strains WWP-2 Hcr⁺ and Hcr⁻ and also *trpA46* (missense) and *trpA96* (ochre) revertants (27). Spontaneous reversion frequencies were decreased approximately 90%, as were 2-aminopurine-induced frequencies. At concentrations of 100 to 150 μg/ml, caffeine is an antimutagen against a variety of spontaneously arising Trp⁺ revertants developing in stationary-phase cells of Trp⁻ ochre, missense, and frameshift mutants of *E. coli* B/r and K-12 (59). The antimutagenic effect of caffeine on Trp⁻ ochre → Trp⁺ revertants was present in both *uvrA⁺* and *uvrA⁻* genetic backgrounds, implying that the antimutagenic mechanism is not an inhibition of excision repair by caffeine.

In the fission yeast *Schizosaccharomyces pombe*, Clarke (23) showed that 1,000 μg of caffeine per ml in the plating medium had an antimutagenic effect on UV-induced Met⁻ → Met⁺ reverse mutations. This antimutagenic effect operated only at lower doses of UV and did not apply to Met⁺ reversions induced by nitrous acid or nitrosomethyl urethane (nitrosomethyl ethylcarbamate). Subsequently, Loprieno and Schupbach (101) showed that caffeine, at 2,000 μg/ml, reduced the yield of UV- and nitrosoguanidine-induced Adn⁺ → Adn⁻ and UV-induced His⁻ → His⁺ mutations in *S. pombe*. There was no detectable effect upon spontaneous mutations in the same systems. Intergenic recombination frenquencies between a *his-2* and a *his-7* mutant were also reduced about threefold when the crosses were performed in the presence of 2,000 μg of caffeine per ml. These results and those of Fabre (51) and Loprieno et al. (100) are consistent with, but do not prove, the hypothesis that caffeine inhibits the activity of a recombinational repair system whose activity is necessary for at least a part of UV and nitrosoguanidine mutagenesis in *S. pombe*, but which is not required for spontaneous, nitrous acid, or nitrosomethyl urethane mutagenesis.

Sarachek et al. (131) have reported an apparent case of caffeine antimutagenesis in the yeast *Candida albicans*. Yields of UV-induced His⁻ → His⁺ reversions were reduced by caffeine under the special circumstances of incubation at 37 C and high UV doses. The effect was found in both caffeine-sensitive and -resistant strains, and revertant yields at 37 C, even in the absence of caffeine, were consistently below those obtained at 25 C.

In view of the findings (119) that caffeine is one of the most effective mutagens and dark-repair inhibitors (42) of several methylated purines tested, it would be extremely interesting to compare the relative antimutagenic activities of a series of methylated purines. If all three activities of methylated purines —mutagenesis of replicating bacterial DNA (57), repair inhibition (65, 144), and antimutagenesis—depend on weak binding to DNA (41), then the same rank order for the various purines might be expected for the above three types of activities. Any deviation from this expectation might well provide clues as to a more specific mode of antimutagenic action of methylated purines.

Manganous Ions

Using a haploid prototrophic strain of *Penicillium chrysogenum*, Arditti and Sermonti (3) showed that the presence of 5 mM manganous chloride in the post-treatment minimal plating medium prevented the appearance of essentially all 8-azaguanine-resistant mutants induced by treatment with the nitrogen mustard methyl-bis(β-chloroethyl)amine. The level of resistance was to 1.5 mM 8-azaguanine, with a dose of the nitrogen mustard giving about 10% survival. Post-treatment incubation of the conidia in a complete medium for 6 h or longer at 24 C, before plating in 8-azaguanine-containing minimal medium on the presence of $MnCl_2$, led to a complete disappearance of the $MnCl_2$ effect on mutational yield. The $MnCl_2$ antimutagenesis did not apply to 8-azaguanine resistance mutations induced by UV light, X rays, diethyl sulfate, or DL-*p*-*N*-di(chloroethyl)phenylalanine. Furthermore, there was no inhibitory

effect of $MnCl_2$ in the plating medium on the yield of nitrogen mustard-induced mutants conferring resistance to 7-azaindole (0.2 to 0.8 mM). The above effects of $MnCl_2$ were possibly related, in some unspecified way, to the earlier findings of Morpurgo and Sermonti (111) that $MnCl_2$ diminished somatic segregation and haploidization and increased the survival of diploid condidia of *P. chrysogenum* treated with nitrogen mustard. Bohme (11) has obtained results in *Proteus mirabilis* indicating that Mn^{2+} ions, in this organism but not in *E. coli* (where Mn^{2+} ions act as a mutagen), cause inhibition of the repair of ethyl methane sulfonate lethal damage. This is probably the result of an effect on the Exr dark-repair system (76), although Mn^{2+} ions caused an enhanced yield of ethyl methane sulfonate-induced Str^R mutations in the *P. mirabilis* system. If so, one might predict that pretreatment of *P. mirabilis* with Mn^{2+} would be antimutagenic against subsequent UV mutagenesis, since UV mutagenesis seems to be totally dependent on the Exr function.

L-Methionine

In the haploid, fission yeast *S. pombe* Clarke (18–20) showed that L-methionine added to the minimal plating medium decreased the yield of spontaneous and HNO_2- or UV-induced $Adn^- \rightarrow Adn^+$ reverse mutations. The antimutagenic effect was optimal with L-methionine concentrations of 20 μg/ml or higher, specific for Adn^+ reversions, and most pronounced for those Adn^+ revertants that were due to suppressor mutations. The inhibitory effect of L-methionine on Adn^+ reversions was paralleled by inhibition of the residual divisions undergone by *adn*$^-$ cells when deprived of extraneously supplied adenine, by an inhibition of complementation between pairs of *adn-1* mutants, and by a decrease in growth of leaky *adn*$^-$ mutants. All of these effects were tentatively attributed to accumulation of *S*-adenosylmethionine in *adn*$^-$ cells in the presence of excess L-methionine. According to this hypothesis, such cells would become depleted of adenosine 5′-triphosphate and thus be unable to express induced mutations. Of some interest would be a demonstration that similar antimutagenic effects of L-methionine occur with *adn*$^-$ mutants of *S. cerevisiae*, and direct measurements of intracellular *S*-adenosyl-methionine concentrations. However, an apparent antimutagenic effect of L-methionine on HNO_2-induced fast-growing Leu^+ revertants of a Leu^- strain, *leu-3*, 241 (20), cannot be explained by this postulated mechanism, although it is possible that it results from a contaminant present in commercially available methionine.

Histidine

Queiroz (126), working with a polyauxotrophic strain of *Saccharomyces cerevisiae*, showed that histidine in the plating medium had a specific antimutagenic effect on tyrosine-inserting class I ochre suppressor (super-suppressor) mutations of UV-induced origin. This histidine effect was relieved by the addition of adenine and could be explained at a biochemical level by the known interactions involving AICAR and feedback inhibition by histidine between adenine and histidine biosynthesis (102). This represents one of the very few cases in which a convincing biochemical explanation exists for a case of antimutagenesis.

Spermine and Other Polyamines, Quinacrine, and Acridines

In a series of papers published over the past decade Sevag, DeCourcy, and their co-workers have presented evidence that the presence of spermine, spermidine, quinacrine (atabrine), and acridine derivatives reduce spontaneous mutability to many different types of antibiotic resistance in a variety of bacteria (35–38, 135–138). These results have, in our opinion, been largely overlooked by geneticists because of the strongly Lamarckian overtones of the explanations provided, including perhaps some confusion over the distinction between a mutational event in the DNA and a mutant cell showing full phenotypic expression. In addition, most of their experiments were based on turbidity measurements in liquid cultures and did not, therefore, measure quantitatively mutation frequencies or rates. Since we do not presently understand the mechanism(s) that generates spontaneous mutations, and since the possible role of repair systems in the antimutagenic effects observed was not examined, it seems important to extend testing of the above-named compounds to mutagenic systems other than spontaneous antibiotic resistance in wild-type bacteria.

To some extent this has already been achieved. Johnson and Bach (80, 81) showed that spermine tetrahydrochloride, at a concentration of 150 μg/ml, caused a small but significant (two- to fourfold) lowering of spontaneous mutation rates. The systems studied were $Str^S \rightarrow Str^R$ in *E. coli* and *Staphylococcus aureus* and $Trp^- \rightarrow Trp^+$ in *E. coli* UC707. Furthermore, $Str^S \rightarrow Str^R$ mutations induced in *E. coli* by growth in the presence of 150 μg of caffeine per ml were reduced about 11.5-fold by the presence

of spermine. These authors also studied UV-induced $Str^S \rightarrow Str^R$ mutations in cells grown prior to irradiation with or without spermine. With a single UV dose of 150 ergs/mm, giving the surprisingly low survival level of approximately 1%, they obtained an approximately 8.5-fold decrease in mutational yield by pregrowing the cells in spermine. This result, however, deserves reinvestigation since the authors did not provide details of the conditions allowed for phenotypic expression of the UV-induced Str^R mutations prior to challenge with streptomycin. Such conditions can dramatically alter the mutant numbers. Caffeine- or UV-induced $Trp^- \rightarrow Trp^+$ reversions also were not investigated in this particular study.

Johnson and Bach demonstrated later (81) that mutations to streptomycin resistance induced in *E. coli* through the action of the Treffers mutator gene (now known to cause A-T → C-G transversions during DNA replication) (33) and 2-aminopurine (causing A-T → G-C transitions) were susceptible to spermine and quinacrine antimutagenesis. These compounds at the concentrations used (150 μg/ml for spermine; 2 to 20 μg/ml for quinacrine) caused approximately 30 and 80% reductions, respectively, in induced mutation rates. Spermine at a concentration of 50 μg/ml had no antimutagenic effect. In the published results, details of spermine antimutagenesis against 2-aminopurine-induced Str^R mutations are not given.

In a later paper, Zamenhof (171), using *E. coli* K-12, showed that spermine tetrahydrochloride (150 μg/ml) and quinacrine hydrochloride (6 μg/ml) depressed spontaneous mutation rates from azide sensitivity to azide resistance and $Met^- \rightarrow Met^+$ reversions about fourfold each. However, these two compounds did not reduce the mutability (in the same genetic background) of the *ast* mutator which had been shown to cause bidirectional transition mutagenesis. Spermine and quinacrine therefore do not appear to be totally nonspecific antimutagens. A detailed knowledge of the mode of action of the *ast* mutator should be helpful in interpreting the failure of spermine and quinacrine to counteract *ast* mutagenesis.

Clarke (27) investigated the influence of spermine (250 μg/ml) on spontaneous and 2-aminopurine-induced $Trp^- \rightarrow Trp^+$ reversions in *E. coli* B/r strain WWP-2 (ochre) in $uvrA^+$ and $uvrA^-$ genetic backgrounds and in *E. coli* K-12 trp^- strains A-46 (missense) and A-96 (ochre). Spermine was not found to be antimutagenic against spontaneously arising revertants in any of the four strains. There was, however, a 25 to 55% reduction in 2-aminopurine-induced Trp^+ revertant frequencies, involving both back mutations and suppressor revertants. Subsequently, Godsell and Clarke (59) investigated the influence of spermine, at 50 to 200 μg/ml concentrations, on spontaneous $Trp^- \rightarrow Trp^+$ reversions occurring in growing and stationary-phase cells. The Trp^- strains used were in *E. coli* B/r and K-12 backgrounds and involved ochre, missense, and frameshift mutations. There was only a weak antimutagenic effect against frameshift revertants in the K-12 strains and against ochre revertants, including suppressors, in the B/r strains. In further tests in this same series of experiments, employing quinacrine at 5 to 30 μg/ml concentrations, Godsell and Clarke found an appreciable antimutagenic effect against Trp^- frameshift revertants in stationary-phase cells, but not against Trp^+ reversions of the other Trp^- strains. Reconstruction experiments were performed and appeared to exclude selection against Trp^+ cells as an explanation for the observed antimutagenesis. Nestmann (117), studying spontaneous and mutator H1-induced T5-resistant mutations in glucose-limited cells of *E. coli* B/r growing in chemostats, found additional evidence for spermine antimutagenesis. In such cells, where mutations arise in a generation time-dependent manner, spermine, at a concentration of 50 μg/ml, caused a 62% reduction in both spontaneous and mutator H1-induced mutation rates.

Nestmann (117) has hypothesized, on the basis of the available biochemical evidence, that spermine antimutagenesis might operate via a stimulation of the exonucleolytic (editing, error-correcting) function of a DNA polymerase (16, 60, 70, 114). For example, Chiu and Sung (16) demonstrated that spermidine strongly stimulates the activity of DNA polymerase B from rat brain but that it does not stimulate the activity of DNA polymerase A from the same source. To the extent that DNA polymerases are involved in repair processes or in error-correcting mechanisms, this type of observation may help to explain the antimutagenic activity of spermidine. Unfortunately, polyamines exert such a diversity of biochemical effects (7, 31, 147, 149) that it may prove extremely difficult to identify the site(s) of polyamine antimutagenesis.

Of obvious interest in the context of polyamine antimutagenesis would be a determination of spontaneous and induced mutation frequencies in bacterial strains that are polyamine auxotrophs (103, 104) when such cells are grown at a range of polyamine concentrations. Such studies might reveal whether or not naturally

occurring polyamines in wild-type microbial cells act as major regulators of mutagenic processes.

Using glucose-limited cells of *E. coli* B/r/1 *trp*$^-$ growing in a chemostat, Webb and Kubitschek (154) showed that in the dark acridine orange at concentrations of 10^{-6} to 5×10^{-6} M caused an approximately threefold reduction in the rate of spontaneous mutations to phage T5 resistance. Similarly, mutations induced by 450 μg of caffeine per ml were reduced to about the same extent by acridine orange. It should be noted that in glucose-limited cultures spontaneous mutation rates are directly proportional to the generation time. (This is in contrast to the situation with slowly growing tryptophan-limited cultures under chemostat conditions [88, 119].)

Magni et al. (105), while studying spontaneous mutations in haploid strains of *S. cerevisiae*, showed that 5-aminoacridine (10 μg/ml) was antimutagenic during vegetative growth. The mutations studied were from canavanine sensitivity to resistance (30-fold reduction) and reversions were from His$^-$ to His$^+$ (sixfold reduction). Unfortunately, no results are reported for the effects of similar concentrations of the agent on spontaneously arising mutations in mitotically dividing diploid cells. In a further study, employing the same canavanine resistance mutation system, Puglisi (124) compared the antimutagenic activity of acridine and three methyl acridines (at 10 μg/ml) on growing haploid cells. Whereas acridine had only a weak antimutagenic effect (50% reduction), the methyl acridines caused up to 60-fold reductions in spontaneous mutation rates. Antimutagenesis by the methyl acridines was highly dependent upon conditions of pH and aeration during growth, but the relatively weak effect of the acridine was not dependent upon these factors.

Ethidium bromide at 2 to 5 μg/ml and hycanthone methane sulfonate at 20 to 40 μg/ml have recently been shown to act antimutagenically in an excision repair-deficient strain of *E. coli* (30, 141). The mutations studied were UV-induced mutations to intermediate- or high-level streptomycin resistance and Trp$^-$ ochre → Trp$^+$ reversions. The Str^R system was chosen since hycanthone is a frameshift mutagen (30). Its antimutagenic activity could be revealed since frameshift mutations are lethal in the streptomycin locus (62, 148).

In view of Riva's (128) demonstration that the frameshift mutagenicity of acridines is not satisfactorily explained on the basis of their intercalating ability, it might be useful to determine whether acridine antimutagenesis is, likewise, unrelated to intercalation. Since reported cases of acridine and spermine antimutagenesis are in replicating systems, it may well be that antimutagenesis results from binding to the DNA polymerase or replication fork complex. Acridines and quinacrine are known to be effective inhibitors of excision and probably other dark-repair processes (30, 53, 158).

Phenothiazine Tranquilizers and Dibenzocycloheptene Antidepressants

An interesting bridge has been created between human pharmacology and microbial antimutagenesis by the findings of Heller and Sevag (66) that tranquilizers and antidepressants can reduce spontaneous mutation frequencies in bacteria. These studies were carried out with *S. aureus* and *E. coli* B and their mutants resistant to streptomycin, sulfathiazole, or chloramphenicol. It will be of considerable interest to determine whether these drugs act antimutagenically against biochemically defined types of induced bacterial mutations and in eukaryotic organisms.

Coumadin (Warfarin)

DeCourcy et al. (36) demonstrated that coumadin inhibited the occurrence of spontaneous streptomycin-resistant mutants of *S. aureus* and *E. coli* and polymyxin-resistant *Pseudomonas aeruginosa*. These results obviously deserve repetition in genetically identified mutational systems involving known types of base-pair changes, and determination of the dependence of the antimutagenic action on DNA replication, repair activities, etc. It may be relevant that coumadin intercalates with DNA.

Actinomycin D and Basic Fuchsin

Puglisi (125) scored spontaneously arising forward mutations to canavanine resistance in a haploid strain of *S. cerevisiae* growing in minimal medium. In the presence of sublethal concentrations of actinomycin D (16 to 48 μg/ml), spontaneous mutation rates were reduced 2.5- to 30-fold. Repeat experiments gave results which agreed within a factor of 2. Likewise, addition of basic fuchsin (10 to 30 μg/ml) to the growth medium caused a marked depression of spontaneous mutation rates to canavanine resistance. However, in the two reported experiments reproducibility of results was very low: 10 μg of basic fuchsin per ml giving in one case a 36.5-fold decrease, but in the other case only a 1.5-fold decrease in mutation rate below the spontaneous rate. In the absence of informa-

tion regarding the mechanism by which the spontaneous mutations are generated in this system and of their molecular nature, any speculation as to the mode of action of the two antimutagens would be premature. However, as pointed out by Puglisi, it is probably significant that both actinomycin D and basic fuchsin bind strongly and weakly, respectively, to DNA and could, therefore, inhibit the production or consequences of single-strand breaks in the DNA.

Chloramphenicol, 5-Hydroxyuridine, 6-Azauracil, Pyronin B on UV-Induced Mutations

UV-irradiated cells of *Salmonella typhimurium* and *E. coli* B/r auxotrophs often, but not invariably, give markedly higher yields of prototrophic revertants when plated, immediately after irradiation, on an amino acid-enriched medium rather than on a minimal medium containing only a low concentration of all specifically required nutrients (155, 156). This enhancement of UV-induced mutational yield by a nonspecific amino acid mixture, also known as the broth effect, is especially pronounced for those mutations to a prototrophic phenotype that result from some classes of ochre or amber suppressor mutations. The broth effect is very dependent on the physiological conditions under which the cells were grown before UV irradiation (155). The broth effect was believed to result from a stimulation of post-irradiation protein synthesis (cf. 10). This in turn caused UV-induced lesions to be removed, not by the error-free excision repair process (called in this context mutation frequency decline [43, 169]) but instead by an error-prone, and hence mutagenic, replicational or post-replicational repair system. Thus, the broth effect may in fact be a composite phenomenon—an inhibition of excision repair (mutation frequency decline) plus stimulation of an error-prone process (10, 22, 55, 63, 109).

Whatever the detailed explanation for the broth effect on UV-induced mutational yield, the enhancing effect of amino acids is abolished by chloramphenicol (168), 5-hydroxyuridine (43), 6-azauracil (44), and perhaps by pyronin B (158). That is, these compounds probably exert their antimutagenic influence because they are acting as inhibitors of protein synthesis. It is of interest to note that 5-hydroxyuridine causes mutation frequency decline in the presence of amino acids only after an initial delay (43). One possibility is that 5-hydroxyuridine must first be incorporated into newly synthesized ribonucleic acid before it can exert its action and inhibit protein synthesis. Although Witkin (158) provided good evidence that pyronin B abolishes the broth effect, this was true only with some batches of the dye. Clarke (26) was unable to show any diminution at all of the broth effect with pyronin B. On the contrary, in these later experiments pyronin B acted as a typical excision repair inhibitor, causing decreased survival of UV-irradiated cells and an increase in mutational yield of a dose-enhancement type. Thus, there may well have been a protein synthesis inhibitor present as a contaminant in some commercial batches of the dye which gave the antimutagenesis (broth effect-abolition) reported by Witkin (158).

An obvious extension of these bacterial studies could be into mutational systems in eukaryotic microorganisms. There is already evidence from the work of Sarachek and Bish (130) that temporary inhibition of protein synthesis after UV irradiation of yeast cells can have an antimutagenic effect, at least when one is dealing with cells in which intracellular amino acid pools have been depleted by prior starvation. Experiments with eukaryotic protein synthesis inhibitors, such as cycloheximide, might well reveal these agents to exert an antimutagenic effect if applied transiently after, for example, UV irradiation of yeast cells.

Genic Derepression

States of genic repression or derepression might be expected to affect observed mutability by a variety of mechanisms (99). First, there might be altered accessibility of the genetic material to direct reactivity with a mutagen. Second, there might be an indirect effect via altered accessibility to repair systems (84) and to mutagenic recombinational processes. Consequently of interest, as examples of physiological antimutagenesis, are several reports that states of genic repression and derepression have marked effects upon mutability and genetic recombination. Brock (13) tested mutability at the β-galactosidase locus when the gene was borne on an F′ episome of *E. coli* K-12 in the presence or absence of the gratuitous inducer isopropyl-β-thiogalactoside. Furthermore, in some of the experiments, lactose i^- (*lacI3*) mutants were tested which were not expected to respond to inducer. The results showed that back-mutations within the lac_z (*lacZ4*) locus were much more frequent in the presence of inducer than in its absence. This was true of back-mutations induced by diethylsulfate or nitrosoguanidine, but not for those induced by gamma irradiation. Although it was claimed that mutagenesis by 2-aminopurine and 5-bromodeoxyuridine did not re-

spond to gene induction, the actual results indicate that under the conditions used (nonreplicating cells) neither of these base analogues is mutagenic. Thus, transcription, or its initiation, was believed in some way to facilitate mutagenesis by alkylating agents. Conversely, one might say that states of genic repression are antimutagenic.

Similarly, Herman and Dworkin (69) studied $Lac^- \rightarrow Lac^+$ reversions in *E. coli* K-12, in this case those induced by the frameshift mutagen ICR-191. They showed that induction of gene expression and specifically transcription and/or translation caused an approximately twofold stimulation of ICR-induced mutagenesis. This was true for several different Lac^- mutants, but one exceptional mutant reverted to Lac^+ approximately sevenfold less frequently when induced. Thus, in agreement with Brock's findings (above), a state of genic repression is usually antimutagenic, except in the one mutant described by Martin and Dworkin.

Savić and Kanazir (133), using *Salmonella hisC* and *hisF* mutants in the presence or absence of an additional histidine operator-constitutive mutation that causes 15-fold derepression of the operon, showed that UV mutability was under repression-derepression control. $His^- \rightarrow His^+$ revertant frequencies were shown to be five- to eightfold higher in derepressed than in repressed genes. Thus, in agreement with the previous studies in *E. coli*, here too a state of genic repression is antimutagenic. One puzzling feature of this study is that a proportion of the His^+ revertants would be expected to be due to suppressor mutations distant from the His operon whose expression was being experimentally manipulated. Equally importantly, Savić and Kanazir showed that the same differential mutagenesis is observed in an excision-repair-defective strain. They have therefore suggested that states of genic repression-derepression affect mutagenesis through altered accessibility to recombinational repair and not to excision repair. This is in line with the finding by Savić (132) in the same *Salmonella* system, that an operator-constitutive mutation (i.e., derepression) caused reduced recombination within the histidine operon. Similarly, Herman (68) showed that gene induction caused locally decreased recombination within the lactose operon of *E. coli*. However, if this were so, then one would have to postulate a rather complex (though not necessarily incorrect) hypothesis to explain why decreased recombination led to increased mutagenesis. (It should be noted that, in the arabinose operon of *E. coli*, Helling [67] reported that occurrence of transcription/translation [derepression] led to *increased* recombination.) Moreover, Shestakov and Barbour (142) found no influence of genic derepression on recombination in the *lac* genes of *E. coli* K-12.

GENETIC ANTIMUTAGENESIS

Genetic Background Effects

There have long been known examples in microbial systems where alleles in one gene had a profound influence on the mutability of another locus (17–19, 58, 168, 172). Some of these cases have been analyzed, particularly where an antimutagenic effect of a second marker was evident. For example, Corran (32) showed that in a $His^- Thr^-$ strain of *B. subtilis* the greatly reduced yield of His^+ revertants, compared to that in the $His^- Thr^+$ strain, was due to the phenomenon of "auxotrophic pre-emption." The excess of $His^- Thr^-$ cells removed threonine from the plating medium and thus prevented the development of $His^+ Thr^-$ cells into colonies. Such an effect could be relieved by excess threonine. In the case of an apparent antimutagenic effect of a Met^- allele upon $Adn^- \rightarrow Adn^+$ reversion frequencies in *S. pombe*, this was shown by Clarke to be due exclusively to the necessary addition of L-methionine to the plating medium (18–20). Similar plating medium effects were excluded by Zetterberg (173) in the case of an antimutagenic effect of a Met^- marker on UV-, dimethyl sulfate-, X-ray-, and nitrosomethylurethane-induced reversion frequencies of $Ura^- \rightarrow Ura^+$ mutations in *Ophiostoma multiannulatum*, and Chopra (17) in the case of an antimutagenic effect of an *adn*$^-$ marker on $Trp^- \rightarrow Trp^+$ reversions of spontaneous origin in *E. coli* B/r.

In the case of an absolute antimutagenic effect of a streptomycin dependence allele upon $Trp^- \rightarrow Trp^+$ reversions induced by UV in *E. coli* (168), this is probably a consequence of reduced efficiency of ochre suppressors (responsible for the Trp^+ phenotype) in the presence of streptomycin-dependent ribosomes (62). A similar but less pronounced antimutagenic effect of streptomycin resistance markers upon UV-induced $Trp^- \rightarrow Trp^+$ reversions (8, 28, 146) has been shown to depend on restricted efficiency of ochre suppressors brought about by the ribosomal mutations conferring streptomycin resistance (2, 56, 75, 148). In these cases the revertant yield could be restored to near normality in the Str^R (suppressor restrictive) strain by the addition of streptomycin to the plating medium (28, 92, 146).

Repair-Deficient Bacteria

There are real difficulties of interpretation in

studies with radiation-sensitive microbial mutants in which the sensitive allele in found to cause depression or elimination of induced mutation frequencies. Such an apparent antimutagenic effect of a radiation-sensitive allele should tants in which the sensitive allele is found to imply that the corresponding wild-type allele necessarily has a positive role to play in mutagenesis by specifying some repair enzyme that has an error-prone, and hence mutagenic, role in some repair process. First, some radiation-sensitive mutations may not be localized in structural loci for repair enzymes. Secondly, absence of mutations may be due to active destruction of premutational lesions, for example, through uncontrolled nuclease activity and excessive DNA degradation, such as occurs in *recA*$^-$ strains after irradiation. With these cautions in mind we shall now outline those cases where mutant alleles, often in known or suspected repair genes, have been found to exert antimutagenic effects.

Witkin (159, 160, 162) has presented good evidence that Exr$^-$ mutants in *E. coli* B or B/r genetic backgrounds are nonmutable by UV light. These are probably equivalent to the Lex$^-$ mutations in K-12, which show similar properties (112). Similarly *recA*$^-$ mutants of *E. coli* have been shown to be totally nonmutable by UV (86, 109, 161). This seems to be true also for gamma rays and thymine deprivation mutagenesis (12). However, both *recA*$^-$ and Exr (Lex) mutants undergo extensive DNA degradation after UV irradiation. It is therefore possible that the reason for the apparent nonmutability of such strains is that "mutations" occur only in dead cells, i.e., that survivors are actually cells that received no irradiation dosage, or at least none that was not rapidly removed by excision repair.

Igali et al. (78) showed that mutability by 9-methoxypsoralen plus 365-nm (long wavelength) UV light was abolished in a *recA*$^-$ or an Exr$^-$ genetic background. The mutations they studied were Trp$^-$ → Trp$^+$ reversions due to a mixture of ochre codon back-mutations and ochre suppressor mutations. Witkin (161, 164) has presented evidence that *recC*$^-$ and *uvrA*$^-$ *recB*$^-$ or *uvrA*$^-$ *recC*$^-$ strains show reduced, but not zero, mutability by UV. However Hill and Nestmann (74) showed subsequently that Lac$^+$ → Lac$^-$ mutations induced by UV are not less frequent in a *recC*$^-$ than in a Rec$^+$ strain. Furthermore, they showed that lethal sectoring led to underestimation of other classes of mutations in *recC*$^-$ strains. It is important, therefore, to decide whether the reduced yields of UV-induced Trp$^-$ → Trp$^+$ and Str^S → Str^R mutations reported by Witkin in *uvrA*$^-$ *recB*$^-$ and *uvrA*$^-$ *recC*$^-$ strains, compared with *uvrA*$^-$ *rec*$^+$ strains, are due to genuine antimutagenesis or to the lethal sectoring artifact. It may be significant that in Witkin's data the frequencies of different phenotypic classes of mutations are apparently reduced to different degrees by the presence of a *recC*$^-$ allele (161). Furthermore, whereas *recB73* and *recC* mutations caused approximately the same degree of apparent antimutagenesis against UV-induced Str^S → Str^R mutations, another *recB* allele, *recB21*, caused a more pronounced diminution in mutational yield (164).

A *polA*$^-$ mutation has been stated to be without any effect upon UV mutability (74, 86, 163, 167). The Kornberg DNA polymerase specified by the *polA* locus is believed to be an integral part of the excision repair process. Nevertheless, this apparent absence of a *polA* effect on UV mutability depends on how the results are expressed. If one plots UV-induced mutation frequencies not against dose but against percent survival (i.e., strains are compared at equal survival levels), then *polA*$^-$ strains show greatly reduced UV mutability. (It should be pointed out that comparison of a Uvr$^-$ (Hcr$^-$) strain with a Uvr$^+$ at equivalent doses would show the Uvr$^-$ to have an apparently greatly enhanced UV mutability (71), whereas when the comparison is made at equivalent survivals the strains show approximately equal mutability (71, 159).) If valid, this comparison at equal survival levels would imply that excision and resynthesis is error prone, whereas the incision step in excision repair is error free (29, 118). This all-important point, as to whether one can validly compare mutation frequencies at equivalent doses of mutagen in pairs of strains showing very different sensitivities to the lethal action of the mutagen, or whether one should rather use comparisons at equivalent survival levels (after different doses of mutagen to the two strains), is not easily resolved (170). It cannot be overemphasized that these two ways of treating the data can give very different conclusions regarding the antimutagenic action of any particular repair-deficient mutant (29). Among factors to be considered in deciding which type of comparison to use, or accept, one might include whether or not mutations and lethal events arise from identical lesions susceptible to the same repair systems and whether mutations occur at random in the population or occur rather in a selected fraction (e.g., survivors, or cells receiving more or fewer than average numbers of lesions) of the population (71, 72). In this connection, one may note cases where survival after mutagenic treatment is not affected but mutations are decreased (e.g., T4

antimutator alleles and HNO_2 or ethyl methane sulfonate mutagenesis) or increased (e.g., UV-induced $Tyr^- \rightarrow Tyr^+$ and $Leu^- \rightarrow Leu^+$ reversions in *E. coli* strain 36-10-45 [24]). Furthermore, there are cases where survival is decreased but mutagenesis is unchanged (e.g., *polA*$^-$ with UV, when compared with *pol*$^+$ at equivalent doses), decreased (e.g., *recA*$^-$ with nitrosoguanidine), increased (e.g., Uvr$^-$ with UV or HNO_2, when compared with Uvr$^+$ at equivalent doses), or totally abolished (e.g., *exrA* or *recA* with UV).

In their extensive study of the effect of Uvr$^-$, *polA*$^-$, and *recA*$^-$ mutations on killing and $Arg^- \rightarrow Arg^+$ mutation induction by a variety of mutagens, Kondo et al. (85, 86) made all of their comparisons of mutational yield at equivalent doses. In Table 1 we compare their results with those obtained if one uses comparisons at equivalent survival levels.

Phage Lambda

There have been a number of reports that repair deficiencies in the host cell exert an antimutagenic action on lambda phage UV mutagenesis, which is normally dependent on irradiation of both phage and host cell. Thus, Miura and Tomizawa (109, 110) showed that UV-induced *c* mutations of λ did not occur in *recA*$^-$ hosts, but that *recB*$^-$ and *recC*$^-$ hosts gave normal yields of such mutants. Phage Red$^-$ and Int$^-$ mutations also failed to impair UV mutagenesis of λ. Subsequently, Defais et al. (39) showed that the Lex$^+$ function of the *E. coli* K-12 host is necessary for production of UV-induced *c* mutations (i.e., Lex$^-$ acts antimutagenically [Lex in K-12 = Exr in B]). Furthermore, in contrast to the earlier report of Miura and Tomizawa (110), they showed that the Red$^+$ function had a small effect on λ UV mutagenesis but a large effect on λ spontaneous mutagenesis. Kerr and Hart (82) also showed that mutagenesis of λ by UV or nitrous acid did not occur in *recA* or Exr$^-$ hosts and were able to separate phage mutagenesis from the UV-reactivation process.

TABLE 1. *Comparison of effects of repair genes at equivalent doses or survivals*[a]

Mutagen	Comparison made at equivalent	Effect of repair gene on $arg^- \rightarrow Arg^+$ frequencies		
		uvr$^-$	*polA*$^-$	*recA*$^-$
UV	Dose	Increased	Same	Abolished[a]
	Survival	Same	Reduced[a]	Abolished[a]
NQO	Dose	Increased	Same	Abolished[a]
	Survival	Same	Reduced[a]	Abolished[a]
MMC	Dose	Abolished[a]	Same	Abolished[a]
	Survival	Abolished[a]	Same	Abolished[a]
X rays	Dose	Same	Same	Abolished[a]
	Survival	Same	Reduced[a]	Abolished[a]
NTG	Dose	Same	Same	Reduced[a]
	Survival	Same	Reduced[a]	Greatly reduced[a]
MMS	Dose	Same	~Same	Abolished[a]
	Survival	Same	Reduced[a]	Abolished[a]
EMS	Dose	~Same	~Same	~Same
	Survival	Same	Reduced[a]	Reduced[a]

[a] Reduction or abolition of $Arg^- \rightarrow Arg^+$ frequencies indicates an antimutagenic action of the Uvr$^-$, *polA*$^-$, or *recA*$^-$ allele. Abbreviations: NQO, nitroquinoline oxide; MMC, mitomycin C; NTG, nitrosoguanidine; MMS, methyl methane sulfonate; EMS, ethyl methane sulfonate.

There is clear evidence from the work of Castellazzi et al. (14) that mutations in the bacterial *recA*, *lexA*, and *zab* (? = *lexB*) loci block UV mutagenesis of phage λ in a *tif-1* host at 42 C. Under these conditions in *recA*$^+$, *lexA*$^+$, Zab$^+$ hosts, Tif-controlled functions, including host functions necessary for mutagenesis of UV-irradiated phage λ, are normally induced. In this connection, it would be extremely interesting to determine whether bacterial *recA*, *lexA*,2 and zab mutations also had an antimutagenic influence on UV-induced mutations taking place in a host cell *mul*$^-$ genetic background (151). It should also be of some interest to determine whether host *recA* and *exr* (*lex*) functions affect UV mutagenesis of other phages, such as T1 or T3, and mutagenesis of λ and other phages by mutagens other than UV and nitrous acid. Pietrzykowska (122), working with 5-bromouracil-induced *am* → *am*$^+$ mutations in phage λ C17*am*08, showed that mutagenesis by this base-analogue depended on host repair functions. Thus, bromouracil mutagenesis of λ was reduced or abolished in the absence of a λ *red* or host *recA* function and in a *lex*$^-$ host.

Tests might also be made in T4 phages of the possible involvement of *v*, *x*, and *y* genes in mutagenesis. To a limited extent this has been done in that Drake (46) found that the *px* component of Harm's *x* mutant (which is actually a *px*, *hm* double mutant) slightly reduces spontaneous mutation rates and decreases T4 UV and methyl methane sulfonate mutagenesis about fourfold. Gene *px* is believed to be involved in generalized error-prone repair.

Antimutator Alleles of Phage T4 Genes 32 and 43

One of the major advances in the understanding of the mechanisms of spontaneous muta-

genesis has been the work by Drake and his co-workers on those alleles in T4 genes *43* and *32* which possess mutator or antimutator activity. Gene *43* is the structural locus for the phage-specified DNA polymerase and gene *32* codes for Alberts' protein (46). Mutations leading to total loss of these functions are lethal, but temperature-sensitive (ts) mutant alleles can be studied (1, 45–49). The antimutator ts gene *43* mutants of phage T4 were shown to depress spontaneous mutations, in a variety of genes, arising by AT → GC transitions. There was no antimutator effect, however, on transversion or frameshift spontaneous mutagenesis. Indeed, some T4 gene *43* ts alleles that were antimutators for transitions actually increased transversion frequencies.

With regard to antimutator effects on induced mutations, transition mutagenesis by 2-aminopurine, 5-bromouracil, or thymine deprivation was strongly suppressed. There was a moderate reduction in mutagenesis by ethyl methane sulfonate at G:C sites and by HNO_2 at A:T sites. In contrast, these particular gene *43* ts alleles had no antimutator effect on hydroxylamine mutagenesis or HNO_2 mutagenesis at G:C sites. It is of some interest that two different antimutator alleles in T4 gene *43*, e.g., ts CB87 (= L141) and ts CB120, had in some cases equal, and in other cases unequal, antimutagenic effects upon the spontaneous or induced reversions of an rII or amber mutant in another gene. This probably indicates that the mutability of a particular base pair is influenced by neighboring base pairs and that such differences are perceived in different ways by the various mutant DNA polymerase molecules (145). Furthermore ts^+ revertants of ts antimutator alleles in gene *43*, with restoration of normal burst sizes at 42.5 C, showed, in some cases, retention of antimutator activity (46). This suggests that some missense mutations in the T4 DNA polymerase allow almost wild-type activity in terms of DNA replication, yet have reduced levels of error-proneness during replication. In the case of T4 gene *32* ts mutants, some caused decreased spontaneous mutation rates at G:C base pairs and in some cases frameshift mutations were also reduced. A temperature-sensitive mutation in the T4 ligase gene has been found by Bernstein and Wilson (9) to exert an antimutagenic effect, in this case against 2-aminopurine- and 5-bromouracil-induced reversions of various amber mutants of T4.

One of the few areas in which antimutagenesis is becoming a biochemically respectable study is in the investigation of mutant T4 DNA polymerases, including those exhibiting antimutator activity. Thus Muzyczka et al. (114) showed that partially purified DNA polymerases from two T4 gene *43* mutator, two antimutator, and one neutral mutant could be distinguished from each other and from wild-type polymerases by their ratios of polymerase:exonuclease:deoxynucleotide turnover activities. Schnaar et al. (134) further showed that polymerases of the antimutator type exhibit a higher exonuclease to polymerase activity ratio than do the wild-type enzymes. Whereas all types of T4 polymerase could discriminate between 2-aminopurine and adenine during in vitro DNA synthesis, the antimutator polymerase of ts L141 incorporated only one molecule of 2-amino purine per 50 molecules of adenine, as compared with values of 1 molecule of 2-aminopurine per 14 molecules of adenine for the wild-type polymerase and 1 per 10 for the mutator polymerase of ts L98. More recently Goodman et al. (61) have shown that antimutator DNA polymerases of T4 are inhibited, in nucleotide incorporation studies, to a far greater extent than wild-type or mutator enzymes by the anticancer drugs adriamycin and daunorubicin and by ethidium bromide and 9-aminoacridine. In contrast, these compounds cause no differential inhibition of polymerase-associated 3'-exonuclease activities of wild-type or mutants.

Rev, Umr, and Rad Mutants in S. cerevisiae

An important advance in understanding the control of induced mutagenesis in eukaryotic microbes has been the isolation and characterization by Lemontt (93–96) of *rev* mutants. These were isolated as showing reduced UV-induced revertability of the *arg4-17* ochre allele. Twenty *rev* isolates could be assigned to three loci, namely *rev1*, *rev2*, and *rev3*. Interestingly the three different *rev3* alleles showed very similar UV survival curves (all *rev* mutants are more UV sensitive than is the wild-type, and *rev2* is synonymous, according to Game and Cox [54], with the *rad5* locus) yet had very different effects on *arg4-17* → Arg^+ revertant yields. Likewise, *rev1-1* and *rev3-1* mutants had a much more pronounced antimutagenic effect on *arg4-17* UV-induced true back-mutational yields than did the *rev2-1* allele. Both *rev1-1* and *rev3-1* greatly reduced UV reversion of the ochre alleles *arg4-17* and *lys1-1* as well as the missense allele *arg4-6*. However, *rev2-1* had much less antimutagenic effect on *arg4-17* or *lys1-1* reversion and no detectable effect on *arg4-6* reversion. One surprising result was that, whereas *rev3-2* and *rev3-3* reduced *arg4-17* true back-mutations induced by UV, these same two

rev3 alleles resulted in apparently increased yields of Arg$^+$ revertants due to suppressor mutations. However, all of these comparisons by Lemontt (93) were made at equivalent UV doses. If one replots his data so as to allow comparisons at equal survivals, then *rev3-1* has the most pronounced antimutagenic effect against UV-induced *arg*$^+$ true back mutations, with *rev1-1*, *rev3-2*, and *rev3-3* having somewhat lesser effects and *rev2-1* being least antimutagenic. Similar comparisons for UV-induced Arg$^+$ revertants due to suppressor mutations show *rev1-1* and *rev3-1* to have the strongest antimutagenic effects, *rev2-1* and *rev3-3* to have intermediate effects, and *rev3-2* to have the least antimutagenic effect. Thus, the degree of antimutagenesis exerted by any given *rev* allele depends upon the particular UV-induced mutation being scored.

Further experiments by Lemontt (96) demonstrated that *rev1-1* and *rev3-1* strongly, and *rev2-1* weakly, suppressed UV-induced forward mutation frequencies from prototrophy to auxotrophy involving many loci throughout the genome. Considering specific forward mutations at the *ade-1* and *ade-2* loci, the same pattern was observed. The rather weak net antimutagenic effect of the *rev2-1* allele was probably due to a counteracting influence of a mutator action of *rev2-1*. Neither *rev1-1*, *rev2-1*, nor *rev3-1* had marked antimutator effects against ethyl methane sulfonate-induced forward mutations from prototrophy to auxotrophy, or specific forward mutations at the *ade-1* and *ade-2* loci.

Further studies by Lemontt (97) resulted in the isolation of 40 Umr$^-$ mutants characterized by their failure to yield UV-induced forward mutations to canavanine resistance. Some of these Umr$^-$ mutants were found to be allelic with *rev* mutants. One awaits with interest further details of the properties of these *rev* and *umr* mutants.

In addition to Lemontt's studies with mutants that were isolated primarily on the basis of their reduced UV mutability, Averbeck et al. (6) have reported on reduced UV mutability in strains selected on the basis of their radiation sensitivity. Mutants r_2^s and $r^s_{3\text{-}1}$ showed greatly reduced yields of Ilva$^-$ → Ilva$^+$ reversions after UV irradiation, compared with wild-type and some other radiation-sensitive strains. Mutant $r^s_{3\text{-}1}$ has been shown subsequently (54) to be located at the *rad-2* locus of *S. cerevisiae*.

Nakai and Yamaguchi (115) studied the induction of a variety of types of mutations in wild-type (with regard to repair) *S. cerevisiae* and the *uvs-1* strain (*rad-1* locus). When comparisons were made on the basis of equal survivals rather than UV dose, *arg4-17* back mutations, Arg$^+$ revertants due to suppressors, and reversions of the *his1-1* frameshift mutation were all induced at lower frequencies in the *uvs-1* than in the wild-type strain (115, Fig. 7).

Resnick (127) showed that in haploid strains of *S. cerevisiae* the *uvs9-3* allele (= *rad-2* locus) (54) resulted in reduced yields of UV-induced Arg$^+$, Lys$^+$, and His$^+$ revertants when comparisons were made between a Uvs$^+$ and the Uvs$^-$ strain at equal survival levels. The reversions studied resulted from presumed addition-deletion (frameshift) and base-pair substitution (transitions, transversions) mutations, with the latter class being represented by back-mutations in structural loci and suppressor mutations. Similarly, Moustacchi (113) studied UV-induced His$^-$ → His$^+$ reversions in Uvs$^+$ and uvs_2 mutants (= *rad1* allele) (54). She showed that when a comparison was made at equal survival levels above about 10%, the uvs_2 strain was less UV-mutable than its Uvs$^+$ parent strain, i.e., that the effect of uvs_2 was antimutagenic. Waters (153) demonstrated that various different *rad-3* alleles result in lowered UV-induced mutation frequencies compared with the *rad*$^+$ strain when comparisons are made at equal survival levels. In this case, the mutations studied were Adn$^-$ → Adn$^+$ reversions and forward mutations conferring resistance to actidione.

Lawrence et al. (91) compared the specificity and frequency of UV-induced reversion of an iso-1-cytochrome *c* ochre mutant in wild-type and radiation-sensitive strains of *S. cerevisiae*. Their results show that the *rad6-1* allele results not only in reduced UV reversion frequencies, but there is also a loss of a high degree of mutagenic specificity, in that the revertants are due to a variety of base-pair substitutions rather than, as in wild-type, predominantly AT → GC transitions in the first position of the ochre codon.

Prakash (123) has also demonstrated that *rad6* and *rad9* mutants greatly reduce chemical and radiation mutagenesis in *S. cerevisiae*. The test system involved reversions of an iso-1-cytochrome *c* mutation. Of numerous mutagens tested, only nitrous acid and nitrosoimidazolidone exhibited apparently normal mutability at low doses in *rad6* and *rad9* strains.

Radiation-Sensitive Mutants in Other Eukaryotic Microbes

Chang et al. (15) and Wohlrab and Tuveson (170) showed that in the *uvs-1* mutant of *Aspergillus nidulans* yields of UV-induced Adn$^-$ → Adn$^+$ reversions were reduced, compared

with those in the Uvs$^+$ strain. This was true whether comparisons were made at equal UV doses or equal survival levels (170). In these same strains, Met$^-$ → Met$^+$ reversions were reduced in the *uvs-1* mutant at doses of UV giving above approximately 3% survival, but at higher doses (lower survivals) the *uvs-1* strain gave apparently greater Met$^+$ yields than the wild-type strain. Similarly, in the *uvs-1* mutant of *Neurospora crassa*, Chang et al. (15) showed that there was a reduced yield of UV-induced mutations to acriflavine or caffeine resistance. Again this conclusion did not depend upon whether one compared the *uvs-1* mutant of *N. crassa* with the wild-type at equal UV doses or equal survival levels. de Serres (40), studying a series of UV-sensitive mutants of *N. crassa* and scoring UV-induced forward mutations at the *adn3A* and *adn3B* loci, showed that *uvs-3* and *uvs-4* gave markedly reduced mutational yields compared with wild-type, and that *uvs-1*, *upr-1*, and *uvs-5* gave smaller antimutagenic effects. In the fission yeast *S. pombe*, Nasim (116) found that the *uvs-1* mutant showed greatly reduced UV mutability. Forward mutations in a series of genes concerned with purine biosynthesis, which converted the phenotype of an *adn-7* mutant from red to white colonies, were scored.

Davies and Levin (34) studied UV-induced mutations from acetate requirement to independence in haploid cells of *Chlamydomonas reinhardi*. They used a wild-type (Uvs$^+$ with respect to dark-repair capacity) and two UV-sensitive (Uvs$^-$) strains in their experiments. Comparisons of mutation frequencies between the Uvs$^+$ and the two Uvs$^-$ strains were made at various doses of UV light. Strain *uvs-1* showed reduced and abnormal revertant frequencies, compared with the Uvs$^+$ strain, giving a near-normal revertant frequency after low doses of UV but a decrease in revertant frequency at higher UV dosages. Strain *uvs-6* showed a revertant frequency that increased with increasing dosages of UV and reached a plateau level. The maximum revertant frequencies given by the two Uvs$^-$ strains were at least 10-fold lower than those given by the Uvs$^+$ strain. Thus, one may conclude that the effect of both Uvs mutations is antimutagenic on UV-induced acetate revertants. This conclusion is confirmed if one replots the results of Davies and Levin (34) so as to allow comparisons to be made of the mutabilities of the Uvs$^+$ and the two Uvs$^-$ strains at equivalent survival levels.

Finally, Arlett (4) provided an intriguing example of a cytoplasmic antimutagenesis. In the red cytoplasmic variant of *A. nidulans*, which showed increased resistance to UV killing but not to gamma radiation lethality, there was total abolition of both UV- and gamma-induced mutations to 8-azaguanine resistance. Such mutations were induced readily in the wild-type cytoplasm. More surprisingly, radiation-induced mutations to 6-methylpurine resistance were induced about normally in the red cytoplasm. Thus, the red cytoplasm exhibits a high degree of antimutagenic specificity. This is reminiscent of the specific antimutagenesis also exerted in *A. nidulans* against 8-azaguanine-resistant mutations by manganous ions (3).

CONCLUSIONS

In concluding this review, there are several points we wish to emphasize. First, it is obvious that knowledge of antimutagenesis is at present very fragmentary. There is clear need to repeat several studies employing mutations involving known types of base-pair changes. Second, it will be of obvious advantage in some instances to test for antimutagenic effects of chemicals in pairs of strains having and lacking a single well-defined repair function. Third, the extension of antimutagenesis studies from phage and bacterial systems into eukaryotic systems is already taking place and will surely increase. Fourth, a word of caution is needed in that the known, or suspected, biochemical effects of many antimutagenic chemicals, e.g., spermine or caffeine, are legion and it will be extremely difficult to pinpoint the one, if indeed it exists, effect which is responsible for their antimutagenic action. Fifth, caution is also necessary in interpreting the antimutagenic effects of radiation-sensitive mutations. Reduction or abolition of mutability in a radiation-sensitive strain need not imply that an error-prone repair gene function is directly implicated in mutagenesis.

Known antimutagens are, like known mutagens, so chemically diverse that a search for new types cannot logically be confined only to a few classes of compounds. If naturally occurring antimutagens are one of the normal regulators of spontaneous and induced mutation frequencies, then one might expect to be able to test this hypothesis by a biochemical analysis of some classes of mutators and mutagen hypermutable mutants. Mutators of several types are well known in several microorganisms (79), and UV hypermutable (*hm*) mutants have been described by Drake (46) in phage T4, and 2-aminopurine hypermutable mutants have been isolated in *E. coli* B/r by Burr and Clarke (unpublished data). To date none of these has been investigated for, or shown to be lacking in, an endogenous antimutagen. At a pragmatic level it would be most useful if antimutagens

should be found which strongly counteract the mutagenic activities of at least some environmental mutagens, particularly those having desirable pharmacological uses.

Antimutagens have already been used, apparently successfully, as adjuvants in chemotherapy of patients in an attempt to reduce the frequency of occurrence of spontaneous antibiotic-resistant bacterial variants (50). It is tempting to speculate regarding whether or not they might also have some practical use in cancer chemotherapy or prevention.

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